potential of mean force (PMF), was calculated along the path. The electrostatic coupling between the excess proton and chloride ion was also explored. These studies therefore provide a more detailed picture of the proton transport process in the ClC-ecl antiporter.

**1713-Pos**

**Ion Selectivity in the Aspartate Transporter $Glt_{PH}$**

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The aspartate transporter $Glt_{PH}$ is an integral membrane protein that catalyses the movement of aspartate across lipid bilayers. $Glt_{PH}$ utilises established ion gradients, transporting two sodium ions with each aspartate molecule. Previous studies have shown that the ion binding sites demonstrate selectivity for $Na^+$ over both $Li^+$ and $K^+$ ($Na^+ > Li^+ > K^+$) [1]. The sodium binding motif is similar to that of another sodium dependent leucine transporter, LeuT. Computational studies have attributed different mechanisms to ion selectivity in each of the two sodium binding sites in LeuT [2]. Selectivity in the first site results from the binding of the negatively charged carboxylate group of the substrate resulting in strong electrostatic interactions while selectivity in the second site is enforced by an almost rigid cavity of coordinating ligands held in place hydrogen bonding networks.

Using various computational techniques, we describe the thermodynamic contributions to the free energy of binding that give rise to the experimentally observed selectivity sequence $Na^+ > Li^+ > K^+$ in $Glt_{PH}$ and compare and contrast them to those in LeuT.

[1] Boudker, O. et al. Nature 2007, 445, 387-393
[2] Noskov, S.; Roux, B. J. Mol. Biol. 2008, 377, 804-818

**1714-Pos**

**Microscopic Mechanism of Ion Selectivity in the Nak Pump**

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The sodium/potassium pump establishes the Na+ and K+ concentration gradients across the plasma membrane of animal cells and therefore plays an essential role in maintaining cell volume and secondary active transport of other solutes. The crystal structures of the Na+/K+ pump provide atomic insight into the binding of K+ ions and conformational transitions during the functional cycle. However, important details about the ion-selectivity remain to be addressed. In particular, 2 out of the 3 binding sites are shared between Na+ and K+ and it is not clear how this pump selects K+ over Na+ when in the outwardly facing conformation (E2P) or Na+ over K+ when in the inwardly facing conformation (E1). We have undertaken free energy calculations to understand the physical principles that govern the ion selectivity in Na+/K+ pump and dissected various factors that may contribute to the selectivity. We found that the pump elegantly modulates the electrostatic environment of the binding sites to achieve the corresponding selectivity. Our results are consistent with available experimental data and provide new hypothesis to test experimentally. [Supported by NIH grant GM062342].

**1715-Pos**

**The Role of Architectural and Structural Forces in Ion Selectivity**

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A novel theoretical framework is presented to clarify the role of architectural and structural forces in ion selectivity by expressing the relative free energy of bound ions in terms of a reduced local system coupled to a potential of mean force (PMF) representing the influence of the surrounding environment. The PMF is separated into two contributions. The first includes all the harsh forces keeping the ion and the coordinating ligands confined to a small microscopic region, but do not prevent the ligands from adapting to ions of different radii. The second regroups all the remaining forces that serve to dictate a precise geometry of the coordinating ligands best adapted to a given ion. In the limit where the precise geometric forces are dominant, the binding site is almost rigid and ion selectivity is controlled by the ion-ligand interactions according to the classic ''snug-fit'' mechanism of host-guest chemistry. In the limit where the precise geometric forces are negligible, the ion and ligands behave as a self-organized ''confined droplet'' that is free to fluctuate and adapt to a smaller ion. But selectivity can also occur under such conditions. In the small and crowded volume, ion selectivity is determined by the ion-ligand and ligand-ligand interactions and is controlled by the number and the chemical type of ion-coordinating ligands. The theoretical framework is used to analyze K+ binding sites in the KcsA channel and Na+ binding sites in the LeuT transporter.

**1716-Pos**

**Mechanisms of Ion Permeation through Gramicidin a Channels**

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Gramicidin A (gA) channels make an ideal system to test all-atom molecular dynamics (MD) of membrane proteins and mechanisms of ion permeation. In addition to being the most studied membrane ''protein'', gA channels are tiny, allowing for long MD runs and calculations of potential of mean force (PMF) in tractable time. The binding sites at either end of the gA channel can both hold a single cation. At low concentration, permeation occurs as a series of independent events in which one cation at a time moves across the pore. Ion permeation usually is described using the ion position z in the direction of the pore axis as a ''reaction coordinate''. But it is not known whether z is a good reaction coordinate to describe the process. A powerful tool to characterize the mechanism of ion permeation in the gA channel is the ''committor'' probability: the fraction of trajectories initiated from a given position that first commit to the left or right binding site of the channel. We evaluate the committor probability distribution function to identify the physical reaction coordinates of a K+ in gA using extensive MD calculations. At high concentration, permeation is dominated by 2-ion processes where cations are bound at either ends of the small pore. To understand the impact of double ion occupancy on the mechanism of ion permeation, we calculate the 2-ion PMF. The results show that if the first ion resides in the inner binding sites at one end of the channel, then the outer and inner binding sites for the second ion at the other end of the channel become shallow. The energetics of double occupancy is explained by considering the dipole moment fluctuation of the single-file water molecules inside channel. [Supported by NIH grant GM070971].

**1717-Pos**

**Thermodynamically Dominant Hydration Structures of Ions and their Role in Ion-Specificity**

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To understand the basis of ion-specific effects in biology, it is necessary to first understand the hydration structure and thermodynamics of ions. Based on a multi-state organization of the potential distribution theorem, we present new insights on the role of ion-water interactions and water density fluctuations at the size-scale of the ion in determining the ion-hydration structure and thermodynamics. We find that the hydration free energy of the ion depends on three quantities: 1) the hydration free energy of the ion in a specified n-coordinate state, where in the n-coordinate state n water molecules are present within the coordination volume of the ion; 2) the probability, xn, of observing that n-coordinate state around the ion; and 3) the probability, pn, of observing n water molecules in the coordination volume in the absence of the ion. Based on this development we find that only a small subset of water molecules in the first hydration shell of the ion sense the chemical type of the ion. Further, these core-water molecules tend to attenuate the interaction of the ion with the rest of the medium, and thus the higher coordination states of the ion more sensitively reflect density fluctuations of the solvent medium at the size scale of the observation volume. The relevance of this development in understanding ion-pairing and the selective binding of ions to biological molecules is discussed.

**1718-Pos**

**On the Domain of Applicability of Currently used Force Fields for the Calculation of the Activity of Alkali Ions at Physiological Ionic Strength**

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Alkali ions are present in virtually all biological processes. Their energetic properties have been so far predicted mostly by MD or MC calculations based on effective potentials derived for infinite diluted conditions (i.e. a single ion surrounding solely by water molecules) [1]. However, in physiological conditions, the concentration of K+ is sub-molar in the cytoplasm [2], and it may be by one, or even two, orders of magnitude larger near globular proteins or nucleic acids and in the active sites of of enzymes or channels [3-5]. The presence of a large ionic strength I is likely to limit the accuracy of the currently used potentials.

Here we will discuss recent calculations of the activity coefficients for K+,Na+ ions at increasing I. Such coefficients have been obtained by calculating the excess chemical potentials from thermodynamics integration [6], with several commonly used biomolecular force fields. Preliminary results show that classical force fields generally overestimate the activity coefficients of ions.

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**1719-Pos**
**Voltage Profile along the Permeation Pathway of an Open Channel**
**Jin Chen**[1], Jorge E. Contreras[1], Albert Y. Lau[2], Vishwanath Jogini[2], Benoît Roux[2], Miguel Holmgren[1].
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In ion channels, the transmembrane potential plays a critical role in ion conduction by acting as a driving force for permeant ions. At the microscopic level, the transmembrane potential is thought to decay non linearly across the ion permeation pathway because of the irregular three-dimensional shape of the channel pore. To experimentally explore the voltage profile of an open channel, we studied the voltage dependence of chemical modification of cysteines substituted along the permeation pathway of cyclic nucleotide-gated (CNG) channels. Because ion conduction through these channels is not sensitive to voltage at maximal open probabilities, nor they desensitize or inactivate when exposed to ligand, CNG channels are an ideal model to these studies. Our functional observations indicate that most of the voltage drop across the permeation pathway occurs along the selectivity filter region of CNG channels. The experimental data are in good agreement with continuum electrostatic calculations using a homology model of an open CNG channel. The focusing of the transmembrane potential across the selectivity filter indicates that the electromotive driving force is coupled with the movement of the permeant ions in the filter, maximizing the efficiency of this process.

**1720-Pos**
**Microscopic Mechanism of Ion Permeation through $K^+$ Channel**
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One of the most basic roles of ion channel is the passive transport of ions through the hydrophilic pore enabling the dehydration of ions. Since the determination of the x-ray crystallographic structure of the $K^+$ channel, many theoretical studies on the ion permeation have been performed. However, the microscopic mechanism of ion permeation, the essence of ion channel, has not been clarified yet.
We study the passive transport of ions through the $K^+$ channel, Kv1.2, by the molecular dynamics simulation in which the electric field is applied. A number of ion permeation is successfully observed. The number of permeated ion, i.e., the channel conductance is mostly proportional to the ion concentration of the bulk. The number of ions in the central cavity also depends on the ion concentration. Although the ions and water molecules are transported alternately, surprisingly, the other manner of ion permeation is also observed. That is, ions can permeate without intervening water molecule at high concentration, on the other hand, a permeating ion is accompanied by two water molecules at low concentration. Therefore, the microscopic mechanism of ion permeation depends on the ion concentration, and the physiological fact that an ion permeates per a water molecule is considered to be the average of these two cases. We also discuss the role of the central cavity by comparing the ion permeation in the channel with that in the model channel.

**1721-Pos**
**Multi-Ion Mechanism of Potassium Channel Rejection of Na and Li Ions**
**Ilsoo Kim**, Toby W. Allen.
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Ion channels catalyze rapid and selective ion movement across cell membranes to control electrical and chemical activity in the body. Potassium channels have the remarkable ability to pass K ions at near diffusion-limited rates, while exquisitely blocking Na ions. The mechanisms of channel selectivity, based on simulation and experimental studies of KcsA blocking by Na and Li ions, will be discussed. Through free energy perturbation and potential of mean force calculations, we find that Na and Li can bind deep into the S4 site of the selectivity filter, coordinated by a plane of four carbonyl oxygen atoms, rather than the usual eight-ligand cage of K. However, we demonstrate that a different multiple-ion mechanism is required for Li or Na ion entry into the filter from the aqueous cavity, involving large energetic barriers that are not encountered by K. We also revisit calculations of the thermodynamic stability of these ions in other sites of the filter, within the framework of a multiple-ion free energy calculation, with some surprising results. We conclude that, under physiological conditions, the rejection of intracellular Na or Li from KcsA occurs upon entry to the filter and is not due to reduced thermodynamic stability at any site inside the filter.

**1722-Pos**
**Exploring the Permeation Mechanism of Valinomycin Across Lipid Membranes**
**Borislava Bekker**, Toby W. Allen.
University of California, Davis, CA, USA.
Valinomycin is a potassium specific ionophore used to transport ions down an electrochemical gradient across lipid membranes. Its small size, high selectivity, and strong antibiotic activity make it an interesting target for molecular dynamics simulations. At the same time its conformational flexibility, which strongly depends on the polarity of its environment, poses a challenge. This work was undertaken in order to elucidate the mechanism of valinomycin mediated potassium transport across a lipid bilayer. We have explored several advanced sampling techniques, but chose to perform multi-dimensional free energy calculations that explore conformational space while computing the potential of mean force for membrane translocation. By computing free energy surfaces with and without a bound $K^+$ ion and calculating the free energetics of the ion binding process, we can describe a cycle that reveals the overall permeation mechanism. Our results using implicit and explicit solvent and membrane models will be discussed.

**1723-Pos**
**Investigating Ion Channels using Chemical Synthesis**
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Chemical synthesis is a powerful method for precise modification of the structural and electronic properties of proteins. The difficulties in the synthesis and purification of peptides containing transmembrane segments have presented obstacles to the chemical synthesis of integral membrane proteins. We will present a modular strategy for the semi-synthesis of integral membrane proteins in which solid phase peptide synthesis is limited to the region of interest, while the rest of the protein is obtained by recombinant means. This modular strategy considerably simplifies the synthesis and purification steps that have previously hindered the chemical synthesis of integral membrane proteins. We will discuss a sandwich-intein fusion strategy and a sumo-fusion and proteolysis approach for obtaining the membrane spanning peptides required for the semi-synthesis. We will demonstrate the feasibility of the modular approach by the semi-synthesis of the $K^+$ channel, KcsA and the non-selective cation channel NaK. The use of chemical synthesis in functional investigations of the KcsA and the NaK channels will also be presented.

**1724-Pos**
**[$K^+$] Induced Conformational Dynamics of the Selectivity Filter of KcsA Monitored by Solid-State NMR**
**Manasi Bhate**, Benjamin Wylie, Lin Tian, Ann McDermott.
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A solid-state NMR study of the selectivity filter of the prokaryotic potassium channel KcsA in a lipid bilayer is presented. The selectivity filter is highly conserved in both bacterial and mammalian channels and chelates $K^+$ very specifically. The selectivity filter is known to exist in many different conformations depending on the identity and local concentration of the permeant ion. Transitions between these different conformations have not been quantitatively characterized in a native bilayer environment. We have used 2D and 3D heteronuclear correlation spectra to site-specifically assign residues in full-length KcsA reconstituted into a lipid bilayer. We report two distinct conformations of the selectivity filter of KcsA in the presence of $K^+$ and $Na^+$. We report significant changes in the chemical shifts of key residues in the filter as the permeant ion is changed from $K^+$ to $Na^+$. Chemical shift analyses using the SPARTA database indicate that the observed conformations are consistent with a $K^+$-bound and a $Na^+$-bound state. Solid-state NMR characterization of both the $K^+$ and the $Na^+$ bound state is important for ensuing studies of channel dynamics, for which, these conformations can be considered limiting structures. Simultaneous detection of both conformers at low ambient $K^+$ suggests that the $K^+$ and the $Na^+$ bound states are in slow exchange on the NMR timescale ($<500\ s^{-1}$).